

Claims

What is claimed is:

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1. A ligand-detection reagent; wherein said reagent comprises a ligand-binding antibody and a ligand analog and a labeling reagent non-covalently bonded to said antibody to form a ternary complex wherein said ligand analog is covalently bonded to a reporter molecule and said labeling reagent comprises a monovalent antibody fragment or a
10 non-antibody protein and a covalently bonded label moiety.

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2. The reagent according to Claim 1, wherein said reporter molecule is selected from the group consisting of a borapolyazaindacene, a coumarin, a xanthene, a cyanine, a fluorescent protein and a phosphorescent dye.

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3. The reagent according to Claim 2, wherein said reporter molecule is selected from the group consisting of BODIPY, OREGON GREEN or fluorinated coumarin dyes.

4. The reagent according to Claim 2, wherein said xanthene dye moiety is fluorinated.

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5. The reagent according to Claim 2, wherein said ligand analog is selected from the group consisting of an amino acid, an enzyme, a kinase substrate, a peptide, a protein, a polysaccharide, a phosphatase substrate, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a cell surface receptor, a drug, a hormone, a lipid, a lipid assembly, a synthetic polymer, a polymeric microparticle, a biological cell and a virus.

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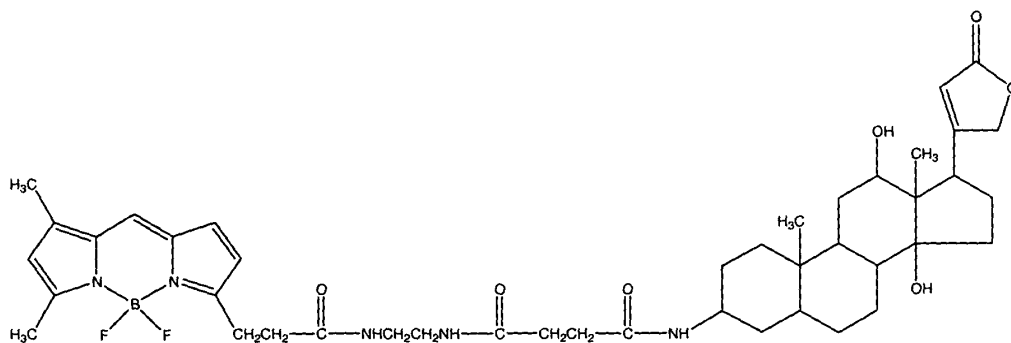
6. The reagent according to Claim 5, wherein said ligand analog is phosphotyramide, phosphotyrosinamide, phosphoserine, phosphoethanolamine, phosphorylated kinase peptide substrate, phosphatase substrate, phosphorylated peptide or digoxigenin.

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7. The reagent according to Claim 6, wherein said ligand analog is phosphotyramide, phosphotyrosinamide, phosphoserine or digoxigenin and said reporter molecule is a xanthene, coumarin or borapolyazaindacene moiety.

8. The reagent according to Claim 7, wherein ligand analog- reporter molecule is selected from the group consisting of Compounds 2, 4-19, 22-29, 31-43,

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and salts thereof.

- 5 9. The reagent according to Claim 5, wherein said monovalent antibody fragment is a Fab or Fab' fragment and is selected from the group consisting of anti-Fc antibody fragment, anti-Fab antibody fragment, anti-kappa light chain antibody fragment, anti-lambda light chain antibody fragment, and a single chain variable protein fragment and wherein said non-antibody protein is selected from the group consisting of a
10 protein G, a protein A, a protein L, a lectin, and a protein G bound to albumin, wherein said albumin is covalently linked to one or more label moieties and albumin is selected from the group consisting of human albumin, bovine serum albumin, and ovalbumin.
- 15 10. The reagent according to Claim 9, wherein said label moiety is selected from a group consisting of a chromophore, a fluorophore, a quenching moiety, a fluorescent protein and a phosphorescent dye.
- 20 11. The reagent according to Claim 10, wherein said label moiety is a fluorophore or a quenching moiety.
12. The reagent according to Claim 11, wherein said fluorophore and quenching moiety are individually selected from the group consisting of cyanine and xanthene moieties.
- 25 13. The reagent according to Claim 12, wherein said monovalent antibody fragment is an anti-Fc Fab fragment.
14. The reagent according to Claim 13, wherein said labeling reagent comprises an anti-Fc monovalent antibody fragment and a xanthene moiety.

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15. The reagent according to Claim 12, wherein said reporter molecule is an energy donor molecule capable of transferring energy to said label moiety that is an energy acceptor molecule wherein an energy transfer pair is selected from the group consisting of Oregon Green 488-Alexa Fluor 555 dye pair, BODIPY-FL-Alexa Fluor 555 dye pair and BODIPY-FL-QSY 9 dye pair.
16. The reagent according to Claim 5, wherein said reagent comprises a ligand antibody, a ligand analog and a labeling reagent to form a ternary complex wherein said ligand analog is selected from the group consisting of phosphotyramide, phosphoserine, phosphotyrosinamide, phosphoethanolamine, phosphorylated kinase peptide substrate, phosphatase substrate and a phosphorylated peptide and said analog is covalently bonded to a xanthene reporter molecule and said labeling reagent is an anti-Fc monovalent antibody fragment covalently bonded to a xanthene labeling moiety or non-fluorescent quenching moiety.
17. A method for determining the presence of a target ligand in a sample, in which is employed a ligand-detection reagent comprising a ligand-binding antibody, a ligand analog and a labeling reagent non-covalently bonded to said antibody to form a ternary complex wherein said ligand analog is covalently bonded to a reporter molecule and said labeling reagent comprises a monovalent antibody fragment or a non-antibody protein and a covalently bonded label moiety whereby the amount of generated detectable signal from said reporter molecule is dependent on the presence of said ligand, said method comprising:
- generating ligand-detection reagent according to any one of Claims 1-16, wherein said ligand-binding antibody, said ligand analog and said labeling reagent are incubated together for a sufficient amount of time to form said complex;
 - incubating said reagent with said sample for a sufficient amount of time for said target ligand to displace said ligand analog from binding groove of said ligand-binding antibody;
 - illuminating said sample with an appropriate wavelength wherein said reporter molecule generates a change in detectable signal in the presence of said target ligand whereby said target ligand is detected.
18. The method according to Claim 17, wherein said target ligand is selected from the group consisting of a phosphorylated biomolecule, kinase substrate, phosphatase substrate, digoxigenin, small molecule drugs, dinitrophenyl, cell surface proteins,

intracellular proteins, extracellular proteins, antibodies, immunogenic peptides, allergens, histamine and cytokines.

19. The method according to Claim 18, wherein said ligand is in solution or immobilized on a solid or semi-solid matrix.
20. The method according to Claim 19 wherein said solid or semi-solid matrix is selected from the group consisting of a membrane, polymeric gel, polymeric microparticle and an array.
21. The method according to Claim 20, wherein presence of said ligand is determined by a shift in color of said detectable signal.
22. The method according to Claim 20, wherein presence of said ligand is determined by an increase in intensity of said detectable signal.
23. The method according to Claim 20, wherein presence of said ligand is determined by a decrease in intensity of said detectable signal.
24. A method for determining the presence of a phosphorylated target ligand in a sample, in which is employed a ligand-detection reagent comprising a ligand-binding antibody that is capable of binding a phosphotyrosine, phosphoserine or phosphothreonine moiety, a ligand analog that is selected from the group consisting of phosphotyramide, phosphotyrosinamide, phosphoserine, phosphoethanolamine, phosphorylated kinase peptide substrate, phosphatase substrate and phosphorylated peptide and a labeling reagent non-covalently bonded to said antibody to form a ternary complex wherein said ligand analog is covalently bonded to a reporter molecule and said labeling reagent comprises a monovalent antibody fragment or a non-antibody protein and a covalently bonded label moiety whereby the amount of generated detectable signal from said reporter molecule is dependent on the presence of said ligand, said method comprising:
 - a. generating a ligand-detection reagent according to any one of Claims 1-16, wherein said ligand-binding antibody, said ligand analog and said labeling reagent are incubated together for a sufficient amount of time to form said complex;

- b. incubating said ligand-detection reagent with said sample for a sufficient amount of time for said phosphorylated molecule to displace said ligand analog from binding groove of said ligand-binding antibody;
- c. illuminating said sample with an appropriate wavelength wherein said reporter molecule generates a change in detectable signal in the presence of said phosphorylated molecule whereby the presence of said phosphorylated molecule is determined.

25. The method according to Claim 24, wherein said phosphorylated target molecule is selected from the group consisting of proteins, peptides, amino acids, nucleotides, phosphatase substrates, and kinase substrates.

26. The method according to Claim 25, wherein said phosphorylated target molecules are immobilized on a solid or semi-solid matrix or are in solution.

27. The method according to Claim 26, wherein said solid or semi-solid matrix is a polymeric gel, a membrane, a polymeric particle, a polymeric microparticle or an array.

28. The method according to Claim 27, wherein presence of said ligand is determined by a shift in color of said detectable signal.

29. The method according to Claim 27, wherein presence of said ligand is determined by an increase in intensity of said detectable signal.

30. The method according to Claim 27, wherein presence of said ligand is determined by a decrease in intensity of said detectable signal.

31. A ligand-detection solution comprising:

- a. a ligand-detection reagent; wherein said reagent comprises a ligand-binding antibody, a ligand analog and a labeling reagent non-covalently bonded to said antibody to form a ternary complex wherein said ligand analog is covalently bonded to a reporter molecule and said labeling reagent comprises a monovalent antibody fragment or a non-antibody protein and a covalently bonded label moiety; and,
- b. a buffer.

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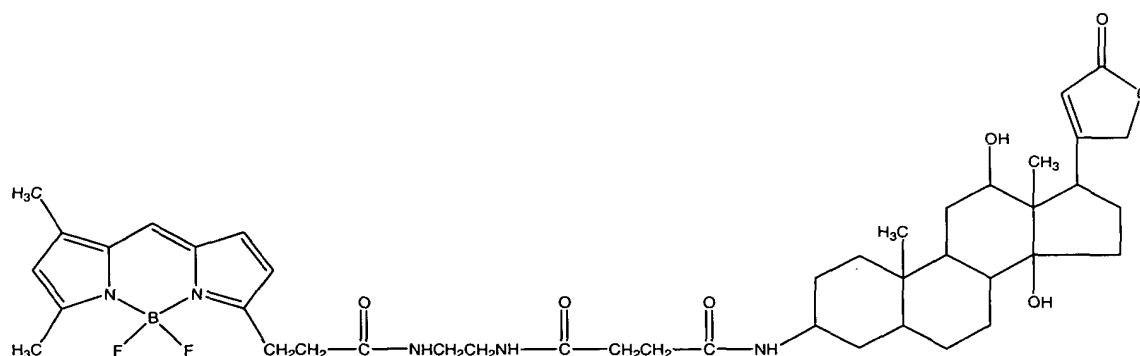
32. The solution according to Claim 31, wherein said reporter molecule is selected from the group consisting of a borapolyazaindacene, a coumarin, a xanthene, a cyanine, a fluorescent protein and a phosphorescent dye.

33. The solution according to Claim 32, wherein said ligand analog is selected from the group consisting of an amino acid, an enzyme, a kinase substrate, a peptide, a protein, a polysaccharide, a phosphatase substrate, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a cell surface receptor, a drug, a hormone, a lipid, a lipid assembly, a synthetic polymer, a polymeric microparticle, a biological cell and a virus.

34. The solution according to Claim 33, wherein said ligand analog is phosphotyramide, phosphoethanolamine, phosphoserine, phosphotyrosinamide, phosphorylated kinase peptide substrate, phosphatase substrate, phosphorylated peptide or digoxigenin.

35. The solution according to Claim 34, wherein said ligand analog is phosphotyramide, phosphotyrosinamide, phosphoserine, phosphoethanolamine or digoxigenin and said reporter molecule is a xanthene, coumarin or borapolyazaindacene moiety.

36. The solution according to Claim 35, wherein said ligand analog is selected from the group consisting of Compound 2, 4-19, 22-29, 31-38,



and salts thereof.

37. The solution according to Claim 36, wherein said ligand analog is Compound 15 or Compound 23.

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38. The solution according to Claim 37, wherein said buffer has less than 5mM phosphate.
39. The solution according to Claim 33, wherein said monovalent antibody fragment is a Fab or Fab' fragment and is selected from the group consisting of anti-Fc antibody fragment, anti-kappa light chain antibody fragment, anti-lambda light chain antibody fragment, and a single chain variable protein fragment and wherein said non-antibody protein is selected from the group consisting of a protein G, a protein A, a protein L, a lectin, and a protein G bound to albumin, wherein said albumin is covalently linked to one or more label moieties and albumin is selected from the group consisting of human albumin, bovine serum albumin, and ovalbumin.
40. The solution according to Claim 39, wherein said label moiety is selected from a group consisting of a chromophore, a fluorophore, a hapten, an enzyme, a quenching moiety, a fluorescent protein and a phosphorescent dye.
41. The solution according to Claim 40, wherein said label moiety is a fluorophore or a quenching moiety.
42. The solution according to Claim 41, wherein said fluorophore and quenching moiety are individually selected from the group consisting of cyanine and xanthene moieties.
43. The solution according to Claim 42, wherein said monovalent antibody fragment is an anti-Fc Fab fragment.
44. The solution according to Claim 43, wherein said labeling reagent comprises an anti-Fc Fab antibody fragment and a xanthene moiety.
45. The solution according to Claim 40, wherein said reporter molecule is an energy donor molecule capable of transferring energy to said labeling moiety that is an energy acceptor molecule wherein an energy transfer pair is selected from the group consisting of Oregon Green 488-Alexa Fluor 555 dye pair, BODIPY-FL-Alexa Fluor 555 dye pair and BODIPY-FL-QSY 9 dye pair.
46. The solution according to Claim 36, wherein said reagent comprises a ligand antibody, a ligand analog and a labeling reagent to form a ternary complex wherein said ligand analog is selected from the group consisting of phosphotyramide,

phosphoethanolamine, phosphoserine, phosphotyrosinamide, phosphorylated kinase peptide substrate, phosphatase substrate and phosphorylated peptide and said ligand analog is covalently bonded to a xanthene or borapolyazaindacene reporter molecule and said labeling reagent is an anti-Fc monovalent antibody fragment covalently bonded to a xanthene label moiety or non-fluorescent quenching moiety.

47. A kit for the detection of a target ligand, wherein said kit comprises a ligand analog, a labeling reagent that comprises a monovalent antibody fragment or a non-antibody protein and a covalently bonded label moiety and optionally a ligand-binding antibody.

48. The kit according to Claim 47, wherein said ligand analog is selected from the group consisting of an amino acid, an enzyme, a kinase substrate, a peptide, a protein, a polysaccharide, a phosphatase substrate, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a cell surface receptor, a drug, a hormone, a lipid, a lipid assembly, a synthetic polymer, a polymeric microparticle, a biological cell and a virus.

49. The kit according to Claim 48 wherein said ligand analog is selected from the group consisting of is a phosphotyramide, a phosphoserine, a phosphotyrosinamide, a phosphoethanolamine, a phosphorylated kinase peptide substrate, a phosphatase substrate, a phosphorylated peptide or a digoxigenin.

50. The kit according to Claim 49, wherein said ligand-binding antibody has affinity for a phosphorylated biomolecule.

51. The kit according to Claim 48, wherein said wherein said monovalent antibody fragment is a Fab or Fab' fragment and is selected from the group consisting of anti-Fc antibody fragment, anti-Fab antibody fragment, anti-kappa light chain antibody fragment, anti-lambda light chain antibody fragment, and a single chain variable protein fragment and wherein said non-antibody protein is selected from the group consisting of a protein G, a protein A, a protein L, a lectin, and a protein G bound to albumin, wherein said albumin is covalently linked to one or more label moieties and albumin is selected from the group consisting of human albumin, bovine serum albumin, and ovalbumin.